

Amendment and Response Under 37 C.F.R. §1.116 - Expedited Examining Procedure

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Serial No.: 09/866,307

Confirmation No.: 4705

Filed: May 25, 2001

For: DNA MOLECULES AND PROTEIN DISPLAYING IMPROVED TRIAZINE COMPOUND DEGRADING ABILITY

### Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

#### Listing of Claims

1-24. (Canceled)

25. (Currently amended) A method for treating a sample comprising an s-triazine-containing compound comprising the step of:

adding a composition to a sample comprising an s-triazine-containing compound,

wherein the composition comprises a protein encoded by a nucleic acid sequence capable of hybridizing under high stringency conditions to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1,

wherein high stringency conditions are hybridization in a buffer containing 0.25 M Na<sub>2</sub>HPO<sub>4</sub> (pH 7.4), 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin, 1.0 mM ethylene diamine tertaacetic acid (EDTA, pH 8) at 65 °C, followed by washing 3x with 0.1% SDS and 0.1x SSC (~~0.1x SSC contains 0.015 M sodium chloride and 0.0015 M trisodium citrate, pH 7.0~~) at 65 °C [(.)],

wherein there is at least one amino acid change in the protein encoded by the nucleic acid sequence as compared with SEQ ID NO:2, and

wherein the protein has an altered catalytic activity as compared to the protein having the amino acid sequence of SEQ ID NO:2,

wherein an altered catalytic activity is selected from the group consisting of improved ability to degrade atrazine and increased degradation of terbuthylazine ~~altered catalytic rate as quantified by  $k_{cat}$  and  $K_m$ , altered substrate range, altered substrate preference, altered activity in aqueous solutions, altered stability in solvents, altered active temperature range, altered salt~~

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concentrations for enzymatic activity, altered pH for enzymatic activity, and improved activity in a soil environment.

26. (Original) The method of Claim 25 wherein the composition comprises bacteria expressing the protein.
27. (Original) The method of Claim 25 wherein the s-triazine -containing compound is 2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine.
28. (Original) The method of Claim 25 wherein the s-triazine-containing compound is 2-chloro-4-(ethylamino)-6-(tertiary butyl-amino)-1,3,5-triazine.
29. (Original) The method of Claim 25 wherein the s-triazine containing compound is (2,4,6-triamino-s-triazine).
30. (Currently amended) The method of Claim 25 wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 5, 6 and 22-26, 22, 23, 24, 25, and 26.
- 31-34. (Canceled)
35. (Previously presented) The method of claim 25 wherein the sample is a water or soil sample.
36. (Currently amended) A method for treating a sample comprising an s-triazine-containing compound comprising the step of:  
adding a composition to a sample comprising an s-triazine-containing compound,

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wherein the composition comprises a protein encoded by a nucleic acid sequence having at least 95% homology to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1,

wherein there is at least one amino acid change in the protein encoded by the nucleic acid sequence as compared with SEQ ID NO:2, and

wherein the protein has an altered catalytic activity as compared to the protein having the amino acid sequence of SEQ ID NO:2,

wherein an altered catalytic activity is selected from the group consisting of improved ability to degrade atrazine and increased degradation of terbuthylazine altered catalytic rate as quantified by  $k_{cat}$  and  $K_M$ , ~~altered substrate range, altered substrate preference, altered activity in aqueous solutions, altered stability in solvents, altered active temperature range, altered salt concentrations for enzymatic activity, altered pH for enzymatic activity, and improved activity in a soil environment.~~

37. (Previously presented) The method of claim 36 wherein the composition comprises bacteria expressing the protein.

38. (Previously presented) The method of claim 36 wherein the *s*-triazine -containing compound is 2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine.

39. (Previously presented) The method of claim 36 wherein the *s*-triazine-containing compound is 2-chloro-4-(ethylamino)-6-(tertiary butyl-amino)-1,3,5-triazine.

40. (Previously presented) The method of claim 36 wherein the *s*-triazine containing compound is (2,4,6-triamino-*s*-triazine).

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41. (Currently amended) The method of claim 36 wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 6 ~~and 22-26~~, 22, 23, 24, 25, and 26.

42. (Previously presented) The method of claim 36 wherein the sample is a water or spoil sample.

43. (Currently amended) A method for treating a sample comprising an *s*-triazine-containing compound comprising the step of:

adding a composition to a sample comprising an *s*-triazine-containing compound,

wherein the composition comprises a protein encoded by a nucleic acid sequence capable of hybridizing under high stringency conditions to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1,

wherein high stringency conditions are hybridization in a buffer containing 0.25 M Na<sub>2</sub>HPO<sub>4</sub> (pH 7.4), 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin, 1.0 mM ethylene diamine tertaacetic acid (EDTA, pH 8) at 65° C, followed by washing 3x with 0.1% SDS and 0.1x SSC (~~0.1x SSC contains 0.015 M sodium chloride and 0.0015 M trisodium citrate, pH 7.0~~) at 65° C [(.)],

wherein there is at least one amino acid change in the protein encoded by the nucleic acid sequence as compared with SEQ ID NO:2, and

wherein the protein has an improved ability to degrade atrazine as compared to the protein having the amino acid sequence of SEQ ID NO:2.

44. (Previously presented) The method of claim 43 wherein the composition comprises bacteria expressing the protein.

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45. (Currently amended) The method of claim 43 wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 6 ~~and 22-26~~, 22, 23, 24, 25, and 26.

46. (Previously presented) The method of claim 43 wherein the sample is a water or spoil sample.

47. (Previously presented) A method for treating a sample comprising an *s*-triazine-containing compound comprising the step of:

adding a composition to a sample comprising an *s*-triazine-containing compound,

wherein the composition comprises a protein encoded by a nucleic acid sequence having at least 95% homology to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1,

wherein there is at least one amino acid change in the protein encoded by the nucleic acid sequence as compared with SEQ ID NO:2, and

wherein the protein has an improved ability to degrade atrazine as compared to the protein having the amino acid sequence of SEQ ID NO:2.

48. (Previously presented) The method of claim 47 wherein the composition comprises bacteria expressing the protein.

49. (Currently amended) The method of claim 47 wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 6 ~~and 22-26~~, 22, 23, 24, 25, and 26.

50. (Previously presented) The method of claim 47 wherein the sample is a water or spoil sample.

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51. (New) A method for treating a sample comprising an *s*-triazine-containing compound comprising the step of:

adding a composition to a sample comprising an *s*-triazine-containing compound,  
wherein the composition comprises a protein comprising an amino acid sequence  
selected from the group consisting of SEQ ID NOS:5, 6, 22, 23, 24, 25, and 26.